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[FROM THE PHYSIOLOGICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY]

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RAPID METHOD OF PREPARING THROMBIN

By W. H. HOWELL

[From the Physiological Laboratory of the Johns Hopkins University]

A FEW years ago the author described a method of preparing pure or approximately pure thrombin. Beginning with a solution of fresh fibrin in strong solutions of sodium chloride the other proteins were removed gradually by repeated shaking of the solution with chloroform. The method is effective, but it is very tedious, requiring usually two months or more when the solution is shaken twice a day. I have modified the method recently so that an equally good, in some respects a better preparation of thrombin may be obtained in three or four days. This method, in detail, is as follows. A large amount of (pig's) fibrin is obtained from the butcher and is washed thoroughly in running water until the hemoglobin is removed. The washing must be done by hand, picking apart the strands that are deeply colored. When sufficiently washed the mass of fibrin is squeezed free from water and is then minced and well covered with an 8 per cent solution of sodium chloride. The preparation is allowed to stand for forty-eight to seventy-two hours in the cold and is then filtered. The filtrate contains thrombin together with other proteins. The filtrate is treated with an equal volume of acetone. A bulky precipitate is thrown down including the thrombin, while a large amount of the other proteins remains in solution. The precipitate is filtered off rapidly through a number of small filters, each containing from 25 to 50 c.c. As soon as the filtrate has run through, each filter is opened upon a pad of filter paper and the precipitate is spread as thin as possible by means of a spatula. Each filter paper, then, with its layer of precipitate is dried rapidly in a current of cold air.

For this purpose I make use of a box with wire trays and an electric fan. When perfectly dry the portion of the filter paper holding the dried precipitate is cut up with scissors into a bulk of water, using an amount of water equal to about two-thirds of the original saline

filtrate. After standing, with occasional stirring, for one-half hour, this solution is filtered. The filtrate contains the thrombin with traces of salt and of a protein coagulable on heating. To remove this latter protein the solution may be shaken with chloroform (10 or 15 c.c. to 100 c.c. of solution). After shaking, the chloroform settles, on standing, as a thick emulsion. This is filtered off and a specimen of the filtrate is tested by boiling. If the specimen shows any opalescence it indicates the presence still of some coagulable protein, and the process of shaking with chloroform must be repeated once or twice until a specimen of the filtrate remains entirely clear on heating. Care must be taken, however, not to continue shaking with the chloroform after the removal of the coagulable protein, as the thrombin also will come down eventually with the chloroform. This result seems to happen much more easily in these solutions practically free from salt than in the strong salt solutions of my first method. To preserve the thrombin finally obtained my method is to evaporate it to dryness in watch crystals, 2 c.c. to a crystal, using the current of air from an electric fan. When thus evaporated the thrombin shows a characteristic snow-flake crystallization together with a few crystals of sodium chloride. The dried thrombin may be kept indefinitely in a desiccator. It is easily and completely soluble in water and shows the reactions described in my previous paper.

To test the action of thrombin it is very convenient to keep on hand specimens of dried oxalated and dialyzed plasma. Dog's blood is oxalated and centrifugalized. The clear plasma is removed and dialyzed in collodion tubes against 0.9 per cent solutions of sodium chloride for twelve to twenty-four hours to remove the oxalate. The plasma is then evaporated in watch crystals, 2 to 5 c.c. to a crystal, in a current of cold air. When dry the plasma may be preserved indefinitely in a desiccator. For use the contents of each crystal are dissolved in 0.9 per cent salt solution, using double the volume of the original plasma. When properly made the dried material dissolves completely, giving a clear solution in which the fibrinogen is in a more normal condition than when obtained by the usual method of repeated fractional precipitation with sodium chloride.

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